The Development of the Pedunculate Spermatophore of a Hermit Crab, *Dardanus asper* (De Haan)[1]

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Although in a few pagurian genera (*Anapagurus*, *Clibanarius*, *Diogenes*, *Eupagurus*, *Pagurus*) the morphological, physiological, and mechanical phenomena associated with the elaboration of the pedunculate spermatophores have been thoroughly investigated, in most pagurian genera these phenomena have been completely neglected.

This paper extends the knowledge of pedunculate spermatophore elaboration and compares the process in *Dardanus asper* (De Haan) with that of other species.

The literature pertinent to the study of the development of pedunculate spermatophores is adequately covered in Mouchet's (1931) bibliography. In so far as can be ascertained, no published work on the development of the spermatophore of *Dardanus asper* has been reported.

Methods and Techniques

Specimens of *D. asper* (Fig. 1*c*) obtained from the Honolulu Aquarium between March, 1949, and November, 1952, were used in this study. These were transported in sea water to the laboratory at the University of Hawaii and used immediately. Cutting the apices of the covering shells (*Tonna perdix*) (Fig. 1*b*) caused the hermit crabs to abandon them quickly. The crabs were then seized, their nerve cords severed, and the dorsal surface of their abdominal wall removed.

The exposed reproductive systems (Fig. 2*c*, *d*) were then freed of the blood vessels and connective tissues which encompassed them. Any macroscopic effects of the asymmetrically placed viscera on the reproductive systems were recorded. Small portions (about 0.5 cm.) of the distal vasa deferentia (Fig. 4*a*) of both the right and left sides were removed and placed immediately in fixative. A label indicating specimen number and side was inserted in each vial so that any dimorphism of the spermatophores could be correlated with that side of the reproductive system most affected by the asymmetric abdomen. The remaining portions of the right and left vasa deferentia and testes were then freed, that of one side was placed in fixative for future sectioning, and the other was dissected. In this manner both right and left testes and right and left vasa deferentia were alternately placed in fixative and alternately dissected.

The testes and vasa deferentia were placed in Bouin's fixative, cleared in toluene, embedded in Tissuemat (54–56°C.) and were serially sectioned at 10 microns. The sections were stained with standard alum-haematoxylin and counterstained with eosin (0.5 per cent solution in 90 per cent alcohol to which 4.0 cc. of 0.1N HCl was added).

Other living right and left vasa deferentia were injected with neutral red (Ehrlich) so that the effects of the muscular contractions on the sperm mass could be more clearly discerned.

All figures were drawn by Evan Gillespie from dissections or from slides prepared by the author.

My appreciation is extended to Mr. Spencer Tinker, Director of the Honolulu Aquarium, who generously supplied the specimens.

Discussion

The abdomen of *D. asper* (Fig. 2*a*), like that of most hermit crabs, exhibits an asymmetrical arrangement of its viscera upon dissection.

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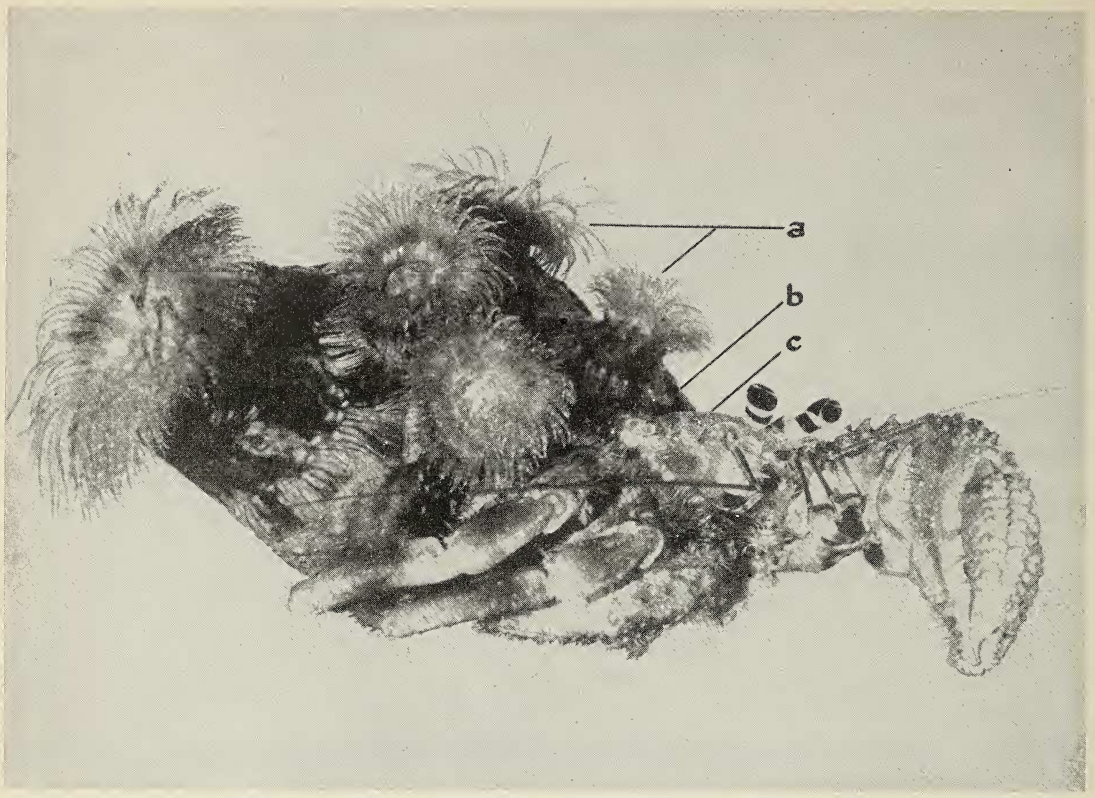

FIG. 1. *Dardanus asper* (DeHaan) (*c*) in a shell of *Tonna perdix* (*b*) encrusted with anemones, *Calliactis armillatas* (*a*). (0.8×.)

The large hepatopancreas (Fig. 2*b*), in following the clockwise coil of the abdomen, carries with it the testes (Fig. 2*c*). Although microscopic dissection is necessary to free the testes of the connective tissue and blood vessels which bind one to the other, they are separate organs not joined by a transverse bridge.

The dissected testis (Fig. 3) discloses numerous sacculi (*a*) through whose thin walls sperm-forming elements (*b*) are clearly discernible. Each sacculus contributes its portion to the continuous sperm mass (*c*) which traverses the length of the testis (*f*). Although differences in the size of the testes are observed, the function of the sacculi is not impaired. Both right and left testes of all the specimens examined during the course of this study were actively producing spermatozoa. There is no sexual season in the male.

Differences in the size of the testes are usually accompanied by differences in the size of the vasa deferentia. However, when right and left vasa deferentia are carefully dissected, both consistently reveal the same fundamental regions (Figs. 4, 5). The distal portion of both right and left vasa deferentia (Fig. 4*a*) are always distended with spermatophores. I have found no evidence of parasitism or dimorphism of the spermatophores.

Although in tracing the development of the spermatophore reference is made to specific cross sections (Figs. 6, 7, 8, 9, 12, 13, 14), serial sections reveal that the morphological changes of the vas deferens are gradual and that these gradual morphological changes are paralleled by physiological changes.

These changes will be discussed as the course of spermatophore development is traced throughout the vas deferens.

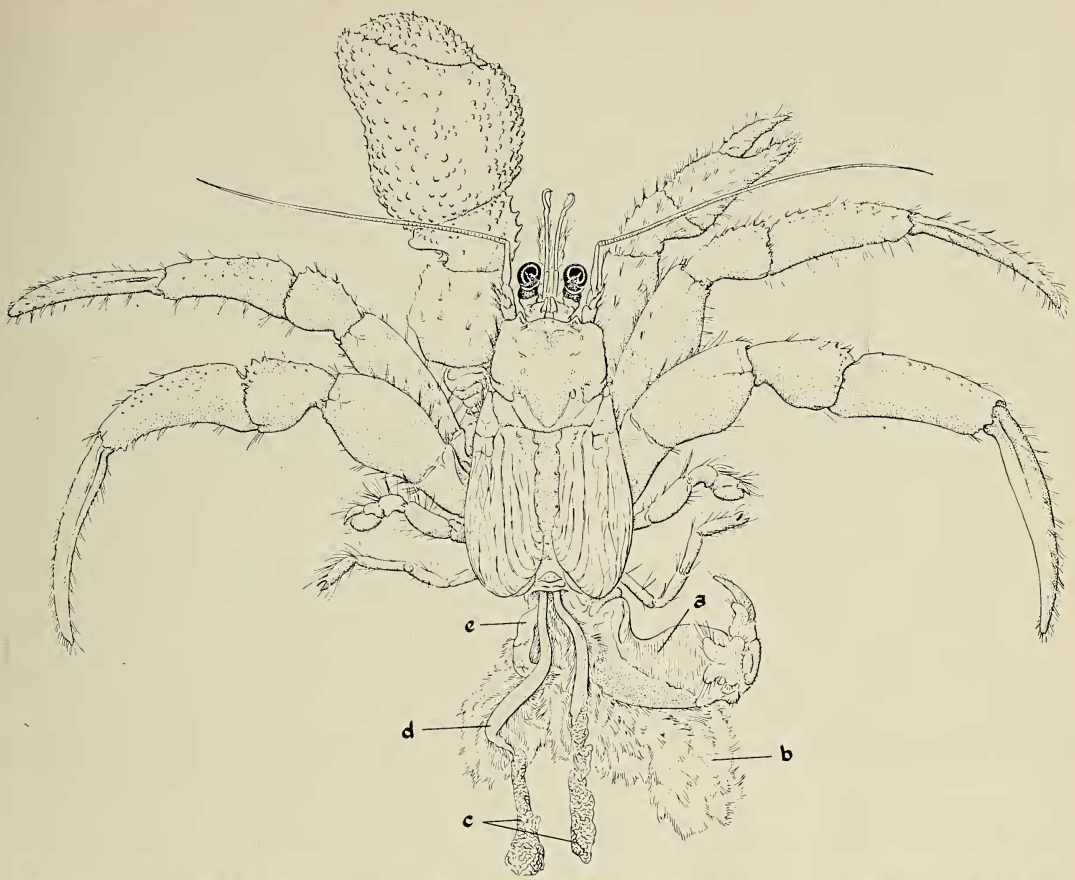

FIG. 2. Dorsal view of dissected *D. asper*. *a*, Asymmetrical abdomen; *b*, hepatopancreas; *c*, testes; *d*, left vas deferens; *e*, intestine. (0.6×.)

Morphology of Living Vas Deferens as Revealed by Dissection

From the comparative morphological study of hermit crab vasa deferentia, Mouchet (1931) concludes that spermatophores with a pedestal and a veil are elaborated in those vasa deferentia which possess two helices. The pedunculate spermatophore of *D. asper* (Fig. 15) which possesses a pedestal (*e*) and a veil (*d*) should, therefore, owe its origin to a vas deferens with two helices. Mouchet (*op. cit.*) also states that the examination of the external form of the hermit crab vas deferens allows one to predict the principal characteristics of the spermatophore that it produces and also to know the method of fragmentation of the sperm column at the moment of its transformation into successive ampullae. She further states that in all hermit crabs it is at the exact point of change of curvature of the two consecutive helices that the continuous flow of sperm is fragmented either into arches or into successive rectilinear fragments.

For those hermit crabs whose vasa deferentia offer little complexity, it may be possible to predict the principal characteristics of the spermatophore and to determine the method of fragmentation of the sperm column, but such is not the case in the highly complex vasa deferentia of *D. asper*. The enlarged portion of the vas deferens of *D. asper* (Fig. 5) reveals that the curvatures (*c*, *d*, *e*) change direction three times before the compact left-handed helix (*g*) is encountered. These coils are referred to as right-handed or left-handed

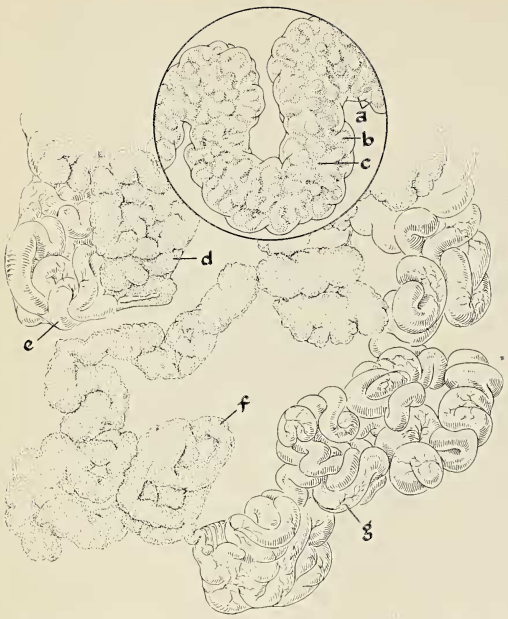

FIG. 3. Posterior portion of the testes. *a*, Sacculi; *b*, sperm-forming elements; *c*, continuous sperm mass; *d*, posterior portion of left testis; *e*, indeterminate portion of left vas deferens; *f*, right testis; *g*, portion of the right vas deferens. (4.5×; insert 10×.)

in relation to the course traversed by the sperm flow; hence the curvatures at *c* are right-handed, at *d* left-handed, at *e* right-handed, and at *g* left-handed. The region *c–e*, with its changes in curvature, was at first included in the indiscriminate region *b* which emanates from the testis. This interpretation must be abandoned as neutral red, injected into the enlarged, distal portion of the vas deferens (Fig. 4*a*), permeates the regions *e*, *d*, *c* (Fig. 5) and reveals a continuous sperm column already encased in its sheath. The change of curvature (*d*) *should* mark the exact location of the fragmentation of the sperm column if Mouchet's thesis is correct. As this fragmentation does not occur until the compact, left-handed coils (*g*) are encountered, Mouchet's assignation of the region of sperm-column sheath formation solely to the right-handed coils of the first helix (*c*) fails to account for any activity in curvatures (*d*, *e*). Obviously, sheath formation and fragmentation are not limited to two consecutive helices in *D. asper*.

The utilization of the dissecting microscope serves not only to reveal the nature of the muscular contractions but also enables one to correlate the changing morphology of the lumen with the complicated molding of the spermatophore.

Cognizant of the arrangement of the oblique muscular fibers in the wall of the vas deferens, one might expect to observe peristaltic waves traversing the tube from the testis distad to the genital pore. These contractions would serve to force the homogeneous sperm mass through a series of ever-changing dies, and, aided by secretions from the epithelial lining of the wall, the heterogeneous spermatophore would evolve. Actual observations, however, prove this supposition to be somewhat inaccurate although correct in principle.

The entire vas deferens, with the possible exception of the enlarged distal portion (Fig. 4*a*), exhibits isolated, intermittent contractions, which persist for well over an hour, even though connective tissue, blood vessels, and nerves are severed. These spasmodic contractions might possibly be attributed to the austere dissection had not this activity been observed in superficial regions of the vas deferens prior to dissection. These muscular contractions are occasionally simple, sphincter-like twitches; however, at times they seem to result in a lateral compression of the wall and at other times in a dorsoventral flattening.

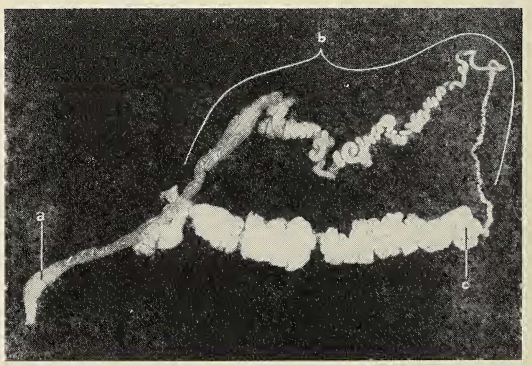

FIG. 4. Dissection of the right testis and vas deferens. *a*, Enlarged distal portion of the vas deferens; *b*, sinuous portion of the vas deferens; *c*, posterior portion of the testis. (1.5×.)

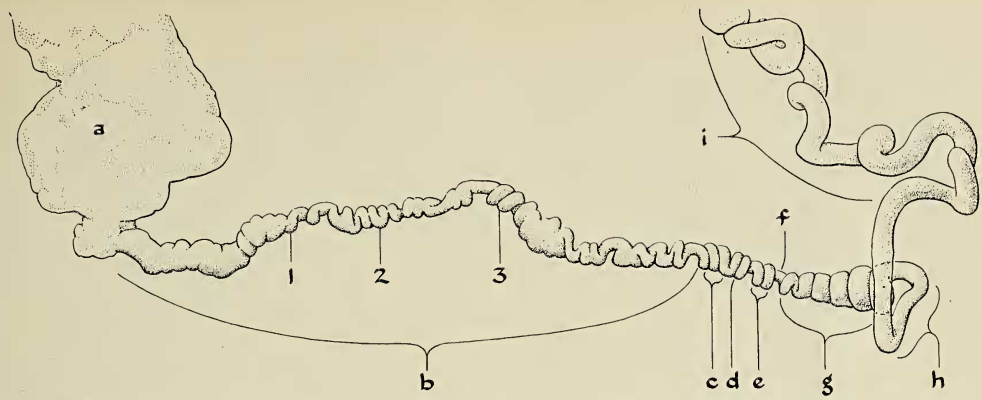

FIG. 5. Dissection of right vas deferens showing exact nature of the coils. *a*, Posterior region of right testis; *b*, (*1*, *2*, *3*) indeterminate region; *c*, first right-handed coil; *d*, first left-handed coil; *e*, second right-handed coil; *f*, region of fragmentation of the sperm column; *g*, compact left-handed helix; *h*, flat spiral; *i*. enlarged, highly contorted region. (6×.)

Even when the internal mass is vitally stained, the result of these many and varied contractions on the formation of the spermatophore is difficult to perceive. However, the internal mass, regardless of what portion of the vas deferens is observed, appears to respond to the ever-changing, restless wall. The combined effect seems not so much to move the mass along as to mold it to an internal die. If the activity which is seen in the altered vas deferens approximates the activity which is obscure in the unaltered vas deferens, spermatophore formation in *D. asper* is a slow and complicated process. During the course of an hour the internal mass rarely moves more than a few millimeters. This mechanical activity, brought about by contraction of the muscular fibers, must be correlated with the morphology and physiology of the vas deferens if the process of spermatophore formation is to be understood.

Correlation between Morphology, Physiology of Epithelial Cells, and Contractions of Muscular Walls of the Vas Deferens

The region of the vas deferens (Fig. 5*b1*) which receives the sperm from the testis presents in cross section (Fig. 6) a tube whose muscle layer (*a*), epithelial layer (*b*), and lumen (*c*) are little specialized. The muscle wall is thin. The cuboidal epithelium is evenly distributed around a cylindrical lumen which contains loose clusters of spermatozoa (*d*). The cuboidal epithelium secretes a substance (*e*) which enters the lumen and mixes freely with the spermatozoa. Whether this substance serves as a nutrient or as a lubricant was not ascertained. The muscular activity, revealed by the dissecting microscope, serves not only to move the sperm mass and to mix it with the epithelial secretion but also to mold the mass in compliance with the cylindrical lumen.

Gradually the internal morphology of the vas deferens changes. From the indeterminate region (Fig. 5*b2*) a cross section (Fig. 7) reveals that the cuboidal epithelium has given way to columnar epithelium (*b*) except at two

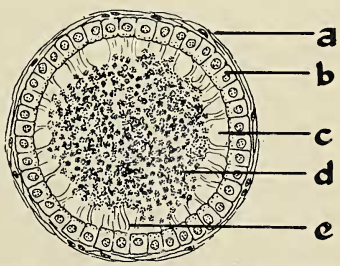

FIG. 6. Cross section through the indeterminate region of the vas deferens (Fig. 5*b1*). *a*, Muscular layer; *b*, cuboidal epithelium; *c*, cylindrical lumen; *d*, loose clusters of spermatozoa; *e*, secretion produced by epithelial cells. (54×.)

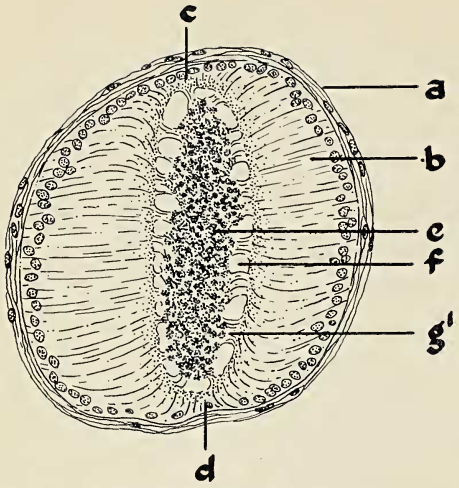

FIG. 7. Cross section through the indeterminate region of the vas deferens (Fig. 5*b2*). *a*, Muscular layer; *b*, columnar epithelium; *c*, *d*, regions where cuboidal epithelium still persists; *e*, sperm column; *f*, elliptical lumen; g^1, secretion produced by epithelial cells. (54×.)

regions (*c*, *d*) which lie directly opposite each other. This change results in an elliptical lumen (*f*) which in the more distal region (Fig. 5*b3*) gradually increases both in width and in height. The columnar epithelial cells (*b*) appear to remain unaltered. Their secretion (g^1) continues but serves less to mingle with the spermatozoa than to remain superficially around the more elliptical and compact sperm mass (*e*). The cuboidal cells (*c*, *d*) have an affinity for the nuclear instead of the cytoplasmic stain. The activity of the muscle wall (*a*) serves to move the sperm mass and to mold it in compliance with the elliptical lumen. There is no evidence as yet of a sperm-column sheath.

From a region still more distad (Fig. 5*c*) a cross section (Fig. 8) reveals that the morphological change in the epithelial layer is even more pronounced. The cuboidal epithelial cells (*c*, *d*) which take up the nuclear stain, now seem isolated between diagonally placed columnar cells (*h*, *i* and *j*, *k*), which likewise take up the nuclear stain. These diagonally placed cells produce a new secretion (g^2), which flows over and covers the distal ends of the epithelial cells (*b*) bounding the elliptical lumen (*f*).

From the region slightly more distad (Fig. 5*d*) a cross section (Fig. 9) reveals that this secretion (Fig. $8g^2$) now lies contiguous with the sperm column. This is the sperm-column sheath (Fig. 9*f*). The phenomena associated with this change of position are obscure but may exist because (1) the sperm column (*d*), due to an increase in size, now presses against the sheath-forming material (*f*); (2) the sheath-forming material (*f*) is freed from the edge of the epithelium (*b*) by a secretion from these cells; or (3) the contractions of the muscular wall (*a*) force the sheath-forming material (Fig. $8g^2$) into contact with the more viscous sperm column (Fig. 9*d*).

Mouchet (*op. cit.*), in describing the sperm-column sheath formation for *Eupagurus bernhardus*, states:

At the two points of maximum depression of the arch [equivalent to areas *c* and *d* of my figure 8], grooves take shape, and in these

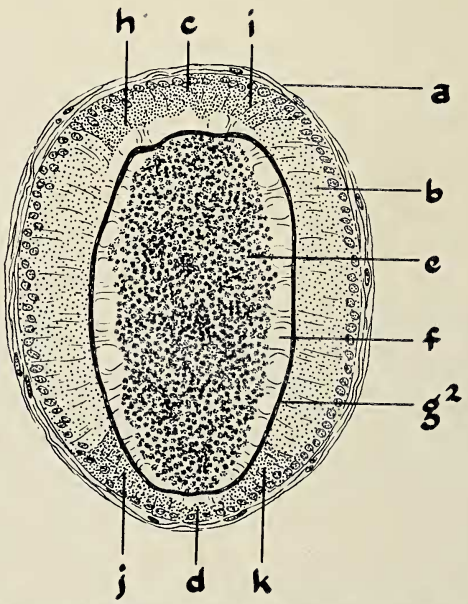

FIG. 8. Cross section through region *c* of the vas deferens (Fig. 5). *a*, Muscular layer; *b*, columnar epithelium; *c*, *d*, regions where cuboidal epithelium still persists; *e*, sperm column; *f*, elliptical lumen; g^2, sheath secretion produced by epithelial cells *h*, *i* and *j*, *k*. (54×.)

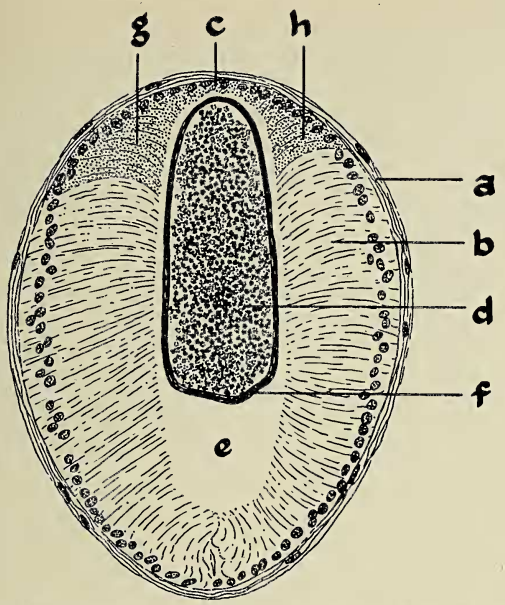

FIG. 9. Cross section through region *d* of the vas deferens (Fig. 5). *a*, Muscular layer; *b*, columnar epithelium; *c*, cuboidal epithelium; *d*, sperm column; *e*, lumen; *f*, sperm-column sheath; *g*, *h*, diagonally placed epithelial cells. (54×.)

grooves a new secretion accumulates, one which lines the interior of the canal and which identifies itself with its basic affinities. . . . Then a continuous cylinder encloses the sperm and it presents two little lateral swellings which seem to glide about in the little grooves . . . in the last turn of the first spindle one of the grooves develops greatly while the other disappears.

It is interesting to note that, although the method of sperm-column sheath formation is quite similar to that of *D. asper*, neither cross sections nor longitudinal sections through this region reveal any lateral swellings associated with the grooves. By the time the sperm column, now enclosed in its sheath, has advanced as far distad as region *e* (Fig. 5), morphological changes of the epithelial cells result in the formation of a pear-shaped lumen. The change in lumen shape, however, is begun in the preceding region (Fig. 5*d*) and can be best explained from a cross section through this region (Fig. 9). The sperm column (*d*), now enclosed in its sheath (*f*), no longer occupies the center of the lumen (*e*) but is nearer one end. At this end the cuboidal cells (*c*) and the diagonally placed columnar cells (*g*, *h*) persist, but those at the opposite end of the lumen (Fig. 8*d*, *j*, *k*) disappear. The only significant difference observed between regions *d* and *e* (Fig. 5) is the shape of the lumen.

When the living vas deferens is injected with neutral red and observed under the dissecting microscope, the sperm column with its sheath appears, in the last right-handed coils (Fig. 5*e*), to be continuous, whereas in the left-handed coils (Fig. 5*g*) it appears to be discontinuous. Interest, therefore, was focused on the region of the vas deferens responsible for the change (Fig. 5*f*). Mouchet (*op. cit.*) describes this differentiation in *Diogenes pugilator* thus:

> Everything happens as if the incurved column of sperm flow, molded by its passage in the first spiral, were maintaining its curving until reaching the second spiral, which is rolled up inversely. Encountering then the wall of the canal on its internal concave face, it hits against it and by successive deflections, describes arcs whose curve is contrary to the canal which contains them. Each point of deflection becomes the extremity of an ampulla in which the spermatozoids of two neighboring half-arches come to accumulate. The slender base of the ampule is formed by the union two by two of the extremities of the arches.

This description is not in accord with the observed process of arch formation in *D. asper*. Likewise, Mouchet's (*op. cit.*) description of the modifications of the process in *Clibanarius misanthropus*, *Eupagurus bernhardus*, *E. prideauxi*, *E. cuanensis*, and *E. hyndmanni* fails to agree with the process in *D. asper*.

The muscular contractions at this region (Fig. 5*f*) appear not unlike those of other regions. Both dorsoventral flattening and lateral compression of the region are observed. This region also shortens and lengthens. The over-all effect of these contractions on the sperm column is comparable to that produced by a tucker attachment of a sewing machine. The continuous sperm column, enclosed in its sheath, arrives at this region and, by the

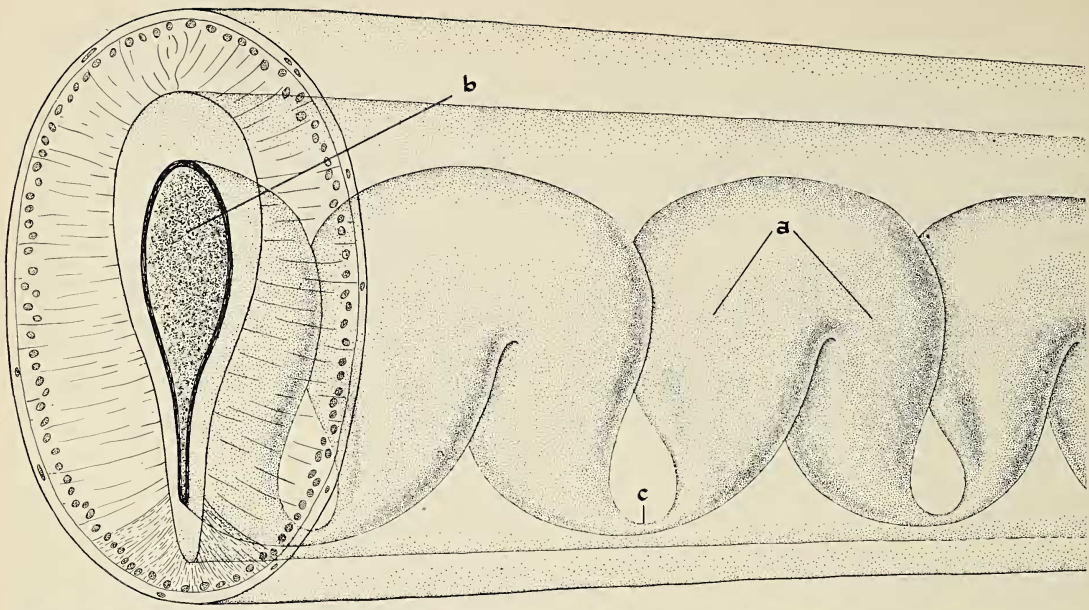

FIG. 10. Reconstruction of the vas deferens through region *g* (Fig. 5). *a*, Ampulla of sperm forming as arch; *b*, pear-shaped sperm column; *c*, connecting sperm-column sheath. (55×.)

contractions of the muscular wall, undulates into continuous sinusoidal curves.

Because of the compact coils of the left-handed helix (Fig. 5*g*), sections parallel to the longitudinal axis of the lumen and at the same time through the plane *c–d* (Fig. 8) are difficult to obtain. Sufficient portions are available, however, to permit a fairly accurate reconstruction (Fig. 10). Although the sperm column appears to be segmented into separate and distinct arches, when viewed through the wall of the vas deferens (Fig 5*g*), these arches are in reality joined one to the other. This obvious discrepancy is easily explained. Throughout the compact, left-handed coils of this region (Fig. 5*g*) the wider portion of the pear-shaped lumen occupies a position nearer the outer edges of the coils, whereas

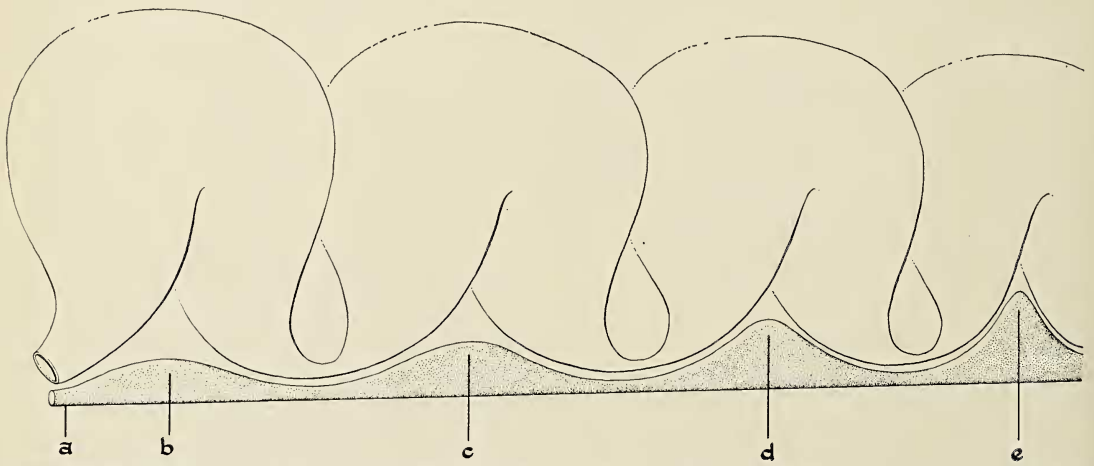

FIG. 11. Reconstruction of the contents of the vas deferens through regions *g*, *h* (Fig. 5). *a*, Continuous thread of stalk material; *b*, *c*, *d*, *e*, stalk-forming material accumulating between the closing arches. (55×.)

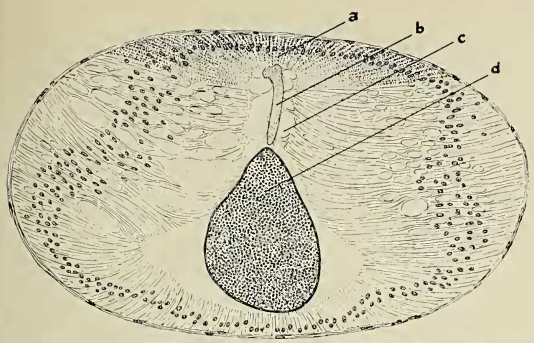

FIG. 12. Cross section through region *h* of the vas deferens (Fig. 5). *a*, Stalk-forming epithelium; *b*, stalk; *c*, deepened groove of lumen; *d*, pear-shaped ampulla. (55×.)

the thinner portion of the pear-shaped lumen occupies a position nearer the inner edges of the coils. The sperm column is molded in compliance with the pear-shaped lumen by muscular contractions of the walls of the vas deferens. When the sperm column is viewed through the wall of the vas deferens, only the thicker portion (Fig. 10*b*) is visible, hence the sperm column appears to be segmented. Each future ampulla of sperm is composed of an arch (10*a*). These are connected by the sperm-column sheath (10*c*). The fate of the connecting sheaths is shown later.

As the last compact coils of the left-handed helix (Fig. 5*g*) are traversed and the region of the flattened spiral (5*h*) is entered, the groove of the pear-shaped lumen deepens. The activity of the epithelial cells at the base of this groove now produces a new secretion. This at first is thread-like, but in the more distal portion of the flat spiral (Fig. 5*h*) thickenings appear. The contents of the dissected, flat spiral which are shown in Figure 11 reveal that this secretion (*a*) accumulates at definite regions (*b*, *c*, *d*, *e*) between the closing arches. The thickenings form short, blunt stumps (*d*, *e*) which are the precursors of the stalks (Fig. 15*c*). The importance of the connecting sheath (Fig. 10*c*) is now apparent. As the closing arches move distad in the deep groove of the vas deferens, the stalk-forming material accumulates only in the regions between the closing arches; the connecting sheaths between adjacent ampullae prevent the stalk-forming material entering the lumen. By muscular contraction of the walls of the vas deferens, the arches finally close, but not until the stalk-forming material has accumulated between them.

In the region distad to the flattened spiral (Fig. 5*i*), a cross section (Fig. 12) reveals that the stalk (*b*) in the deep groove of the lumen (*c*) lengthens both by continued secretion by the epithelial cells (*a*) and by the muscular activity of the walls of the vas deferens.

The lengthening of the stalks carries the ampullae of sperm "aloft," and the connecting sperm-column sheaths between adjacent ampullae become extremely thin and finally obscure.

As this lengthening process continues, a more distal cross section (Fig. 13) reveals that the epithelial cells (*c*), which line the deepened groove of the lumen, produce still another

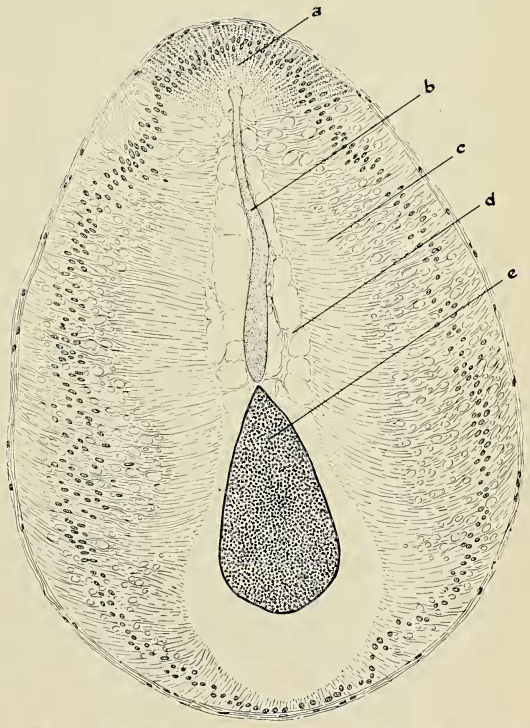

FIG. 13. Cross section through region *i* of the vas deferens (Fig. 5). *a*, Stalk-forming epithelium; *b*, stalk; *c*, veil-producing epithelium; *d*, veil-producing secretion; *e*, ampulla of sperm. (55×.)

secretion (*d*). This secretion is the precursor of the veil (Fig. 15*d*). As the vas deferens is traversed, this secretion, molded by the lateral contractions of the muscular wall, surrounds the stalks (Figs. 13*b*, 15*c*). The secretion from the diagonally placed epithelial cells at the base of the deep groove forms the viscous pedestal (Figs. 14*b*, 15*e*).

The deep groove, so characteristic of the lumen, is no longer present in the enlarged, apical portion of the vas deferens (Fig. 4*a*). For a considerable distance the vas deferens presents a cylindrical lumen. Gradually two folds appear in the epithelium. From these typhlosole-like folds (Fig. 14*c*) a new secretion (*f*) encompasses the completed spermatophores.

Conspicuous longitudinal muscles (Fig. 14*a*) serve to move the completed spermatophores toward the genital pore. When the spermatophoric mass is first extruded, it is difficult to distinguish the pedestal from the veil. It is the viscous pedestal, however, which allows the spermatophore to become attached; the veil is not sticky.

Neither the pedestal nor the veil is segmented. The spermatophore of *D. asper* is, therefore, a continuous ribbon, or two ribbons if both vasa deferentia expel their contents simultaneously.

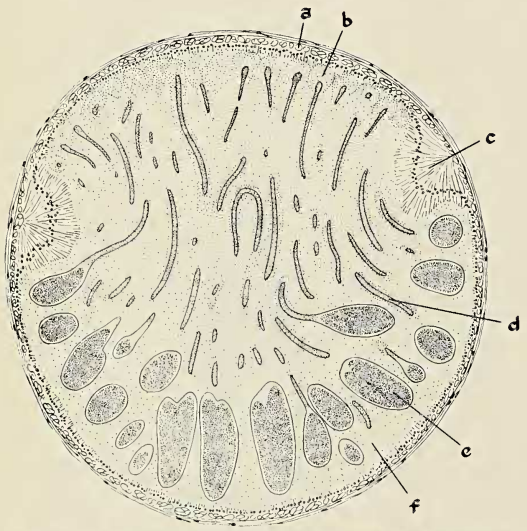

FIG. 14. Cross section through the apical region of the vas deferens (Fig. 4*a*). *a*, Longitudinal muscle layer; *b*, pedestal of a single stalk; *c*, "typhlosole"; *d*, portion of a stalk; *e*, ampulla of sperm; *f*, mucus. (42×.)

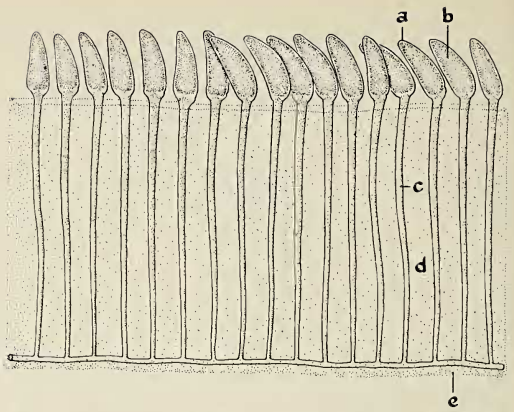

FIG. 15. The completed spermatophore. *a*, Sheath of ampulla; *b*, spermatozoa; *c*, stalk; *d*, veil; *e*, pedestal. (35×.)

Comparison of Regions of Spermatophoric Development in D. asper *with Those of Other Species*

Mouchet (*op. cit.*) assigns nine regions of activity to the vas deferens of *Diogenes pugilator* Roux. The spermatophoric differences observed in other species are attributed to vasa deferentia which lack one or more of these regions. The following are the nine regions of activity assigned to the vas deferens of *D. pugilator:*

1. A rectilinear, irregular part of the canal coming from the testis.

2. A right-handed helix, where the canal narrows, in the last turns of which the shell of the ampulla is secreted.

3. A left-handed helix, where the canal increases in diameter, in the first turns of which the column of sperm is fragmented into segments by its being curved into small arches and by ampullae being formed by the uniting of their extremities.

4. In the last turns of the second helix each ampulla acquires a short and thick stalk.

5. The internal canal enlarges into a spindle-shaped chamber. At the beginning of the spindle, in the thin part, the secretion of the

TABLE 1
THE PRESENCE, ABSENCE, OR MODIFICATION IN OTHER SPECIES OF CRABS OF THE NINE REGIONS OF THE VAS DEFERENS PROPOSED FOR D. PUGILATOR

SPECIES	REGION								
	1	2	3	4	5	6	7	8	9
Diogenes pugilator	p	p	p	a	p	a	p	p	p
Eupagurus bernhardus	p	p	p	a	p	a	p	p	p
Eupagurus prideauxi	p	p	p	a	p	a	p	p	p
Eupagurus cuanensis	p	p	p	a	p	a	p	p	a
Anapagurus hyndmanni (left side)	p	p	p	a	p	a	p	p	p
Anapagurus hyndmanni (right side)	p	p	p	a	p	a	a	a	p
Clibanarius misanthropus	p	m	m	m	m	a	a	a	p
Pagurus arrosor	p	m	p	p	p	p	a	m	p
Dardanus asper									

p = presence; a = absence; m = modified.

pedestal is produced in the form of a continuous ribbon which upholds the individualized stalks.

6. In the thick part of the spindle the stalks are stretched.

7. At the distal extremity of the spindle, the segmentation of the ribbon of the pedestal takes place.

8. In the tube which comes after the spindle, the spermatophores are in single file in the canal, the ampullae directed proximally.

9. The canal becomes larger, the ampullae dispose themselves in any way, the pedestals staying in rank, one after another, until they reach the genital aperture. At the beginning of this region the gland cells secrete a mucus which surrounds the spermatophores.

The presence, absence, or modifications of these nine regions in the species so far adequately investigated are summarized in Table 1. *Dardanus asper*, although possessing region 1, does not possess a well-defined region 2 in which the coils of a right-handed helix secrete the sperm-column sheath. In this respect the vas deferens of *D. asper* resembles *Pagurus arrosor* in which a fine tube of consecutive, closed turns rotates now to the right and now to the left. *D. asper* resembles *Eupagurus bernhardus*, *E. prideauxi*, *E. cuanensis*, *Anapagurus hyndmanni*, *Clibanarius misanthropus*, and *Diogenes pugilator* in that the sperm column is segmented between regions 2 and 3. *Dardanus asper* resembles *Diogenes pugilator* in possessing region 4, where the stalks appear, region 5, where the pedestal is developed, and region 6, in which the stalks are lengthened, but differs from *Diogenes pugilator*, *E. bernhardus*, *E. prideauxi*, *E. cuanensis*, and *A. hyndmanni* in not having region 7, where the pedestal is segmented. Although *D. asper* possesses region 8, in which the spermatophores are in single file, the ampullae are not necessarily directed toward the beginning.

In so far as can be determined, *D. asper* is the only hermit crab which has been investigated in which the stalks lengthen, but the pedestal fails to segment. The spermatophore of *D. asper* may appear sufficiently different from those previously described for other hermit crabs to set it apart in the scheme of spermatophoric development. However, too few genera have been investigated to draw any valid comparisons either on the complexity of their vasa deferentia or the spermatophores which they produce. In all hermit crabs studied the spermatophore is developed with great precision. It is always possible to determine the species by careful examination of the spermatophore. The effects of a changing environment on the form of the spermatophore should prove in *Coenobita* and *Birgus* a lucrative field for further investigations.

RÉSUMÉ

The effect of crowding on the reproductive

organs is negligible, as spermatophores produced by right and left vasa deferentia reveal no dimorphism.

The living vasa deferentia, freed of the concealing testes, exhibit spasmodic contractions. These serve not only to move the sperm mass but to mold it in compliance with the gradually changing internal die of the vasa deferentia, i.e., first cylindrical, then elliptical, then pear-shaped. Serial sections reveal that these gradual morphological changes are paralleled by physiological changes.

A continuous sperm mass emanates from the testis and enters the undifferentiated portion of the vas deferens. Here the epithelial cells secrete a substance of unknown function which mixes with the spermatozoa.

Epithelial cells, isolated at opposite ends of the elliptical lumen, produce a new secretion which at first covers the distal ends of the epithelium but later comes to lie contiguous with the sperm column. This is the sperm-column sheath.

The sperm-column sheath is formed through several regions of the vas deferens, not solely in the coils of a right-handed helix.

Longitudinal sections through the region of the last compact, left-handed helix fail to show the fragmentation of the sperm column into separate ampullae. The sperm column remains continuous, but by muscular contractions of the wall of the vas deferens it is forced into continuous sinusoidal curves or arches.

The groove of the now pear-shaped lumen deepens, and a new substance is secreted from the epithelial cells at its base. This is at first thread-like but later thickens in the regions between the closing arches. These accumulations are the precursors of the stalks. The muscular activity which closes the arches to form the ampullae of sperm is co-ordinated with that which lengthens the accumulations of this secretion to form the stalks.

Cross sections through the region of the flattened spiral reveal the continued lengthening of the stalks and show the origin of a new secretion, the precursor of the veil. As the lumen of the vas deferens increases in diameter, the veil and the stalks become more elongate, and the ampullae of sperm are borne on the distal ends of the stalks. The sperm-column sheaths between adjacent ampullae are stretched by this process. They become extremely thin and, finally, are no longer detectable, at least not with the staining technique employed.

Cross sections through the enlarged, apical portion of the vas deferens reveal a cylindrical lumen distended by fully formed, pedunculate spermatophores. However, two typhlosole-like folds develop which produce a mucoid secretion which encompasses the spermatophores.

Muscular contractions of the wall of the vas deferens serve now to expel the completed spermatophore. When first expelled, it is difficult to distinguish the pedestal from the veil. If the spermatophore of *D. asper* is allowed to stand in tap water, the veil soon disintegrates, leaving the upright stalks with their ampullae directly attached to the continuous, unsegmented pedestal. *Dardanus asper* is the only hermit crab so far studied whose vasa deferentia possess the region for the lengthening of the stalks but lack the region for the segmentation of the pedestal.

REFERENCE

MOUCHET, S. 1931. Spermatophores des crustacés décapodes, anomures et brachyures et castration parasitaire chez quelques pagures. *Sta. Océanogr. de Salammbô, Ann.* 6: 1–203.